5 INTERNAL CONTROLS AND STANDARDS - POLYMERASE CHAIN REACTION (PCR) PROCEDURES

Analysis of evidence samples will be conducted to provide the maximum information with the least consumption of the sample. This requires the use of critical judgment and the use of standards and controls. At each step of the testing procedure standards and controls must be used to evaluate the effectiveness of the testing process and to assure that the procedure is being properly performed. Refer to the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section III, Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 16 BIO System for the interpretation guidelines of the following controls.

5.1 Reagent Blanks

The reagent blank is a check for possible contamination of the reagents by human DNA. Testing of the reagent blank is performed by carrying out the DNA extraction in a tube containing no DNA, just reagents. This blank extract is then amplified and analyzed on a typing gel. Reagent blanks should produce no results.

5.2 Plate Blanks

The plate blank is a check for possible contamination from well-to-well during the DNA isolation step using the BioMek 2000 Automation Workstation. Testing of the plate blank is performed by carrying out the DNA extraction in a well containing no DNA, just reagents. This blank extract is then amplified and analyzed on a typing gel. Plate blanks should produce no results.

5.3 Random Sample (used during the analysis of convicted offender and arrestee samples)

A random sample will be run with each set of convicted offender and arrestee sample extractions to serve as a verification that the samples are successfully being entered into the Combined DNA Index System (CODIS) and the search algorithm is working properly. The random sample is a previously analyzed convicted offender sample for which the identity of the sample is known to the Forensic Biology Program Manager. This sample serves as an internal laboratory control since the DNA profile is not known to the DNA Data Bank analyst and must be verified by the Forensic Biology Program Manager or designee prior to the sizing data being considered acceptable. If a sample must be re-extracted a new random sample must be extracted along with the sample.

5.4 Procedures for Estimating DNA Recovery

A procedure must be used for estimating the quantity of human DNA recovered from the evidence sample.

5.4.1 AluQuant™ Human Quantitation System

The AluQuant™ system utilizes a solution hybridization approach to generate light (luciferase reaction) proportional to the amount of human DNA present which can be quantitated on a luminometer. Human DNA can be accurately estimated in the range of 20pg/µL to 4 ng/µL in the sample being analyzed.

5.4.2 Yield Gel

The yield gel procedure for assessing the quality and quantity of isolated DNA will not be used for casework samples in place of the AluQuant™ procedure for assessing the quantity of isolated DNA. However, the yield gel may be used on known reference samples and utilized to evaluate the need for dilutions of casework samples believed to contain a high concentration of DNA. A set of high molecular weight DNA calibration standards is used on the yield gel to permit a quantitative estimate of the amount of DNA recovered (i.e., 5 to 500 ng). This procedure provides an estimate of the quantity of DNA recovered and the degree of DNA degradation.

5.5 Negative Amplification Control

The negative amplification control is a check of the PCR amplification reagent for contamination that may have occurred during the set up of the PCR reaction.

5.6 Positive Amplification Control(s)

The positive control (K562 or GM9947A Cell Line) is DNA of a known profile and is used to monitor the reagents used during the PCR reaction, the accuracy of the thermal cycler, and as a typing gel migration control.

5.7 Procedure for Estimating the Extent of Amplified Product

A product gel should be used for estimating the extent of amplified product. The amplified DNA is then compared against a standard molecular weight ladder or internal lane standard.

♦**END**